Response of *Acer saccharum* Seedlings to Elevated Carbon Dioxide and Ozone[1]

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ABSTRACT. **Newly germinated seedlings of *Acer saccharum* were grown in atmospheres of elevated carbon dioxide (CO_2) or ozone (O_3) for 85 days. Net photosynthesis measured on initial leaves and recently formed leaves tended (though not always statistically significant) to increase with an increase in CO_2. Biomass measured at the end of the study also increased with an increase in CO_2. Ozone at 0.15 ppm did not have a significant impact on either net photosynthesis or growth; however, with O_3-treatment, biomass increased at elevated CO_2 levels.**

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INTRODUCTION

The atmosphere is currently undergoing significant changes primarily as a result of human activities such as combustion of large amounts of fossil fuel. Effects of such use of fossil fuels include elevation of the level of carbon dioxide (CO_2) in the atmosphere. The concentration of this gas (an important "greenhouse gas") has increased by approximately 25% since 1950 to the current level of nearly 360 ppm, and projections suggest it will double within the next 50 to 100 years (Schneider 1989). Another anthropogenic-related change occurring in the atmosphere is the elevation of ozone (O_3) concentrations.

Currently there are inadequate experimental data to accurately predict the response of forests to these atmospheric changes (Allen 1990). A review of the literature would suggest that increasing CO_2 levels should increase forest ecosystem productivity; whereas, increasing O_3 concentrations would most likely cause reduced productivity. The objective of the present study was to investigate the response of *Acer saccharum* to concurrent increases in the levels of CO_2 and O_3. This investigation examined the photosynthetic and growth response of newly germinated seedlings grown in elevated CO_2 and O_3.

MATERIALS AND METHODS

Newly germinated seedlings of *Acer saccharum* were placed in 15 cm plastic pots in a non-soil commercial potting mix approximately two weeks after germination. Nine seedlings were immediately placed in each of eight fumigation chambers constructed from 0.63 cm Plexiglas™. The chambers were 45.7 cm square by 76.2 cm high and were located inside environmental growth chambers for temperature and light control. Each fumigation chamber contained a circulating fan to mix the air in the chamber, and a gas exchange system that allowed the air in the chambers to be replaced at approximately 1-minute intervals. The seedlings were grown under a 16-hour photoperiod and a light level of 300 umol m^{-2} sec^{-1}. The day-night temperature regime was 25° C day and 20° C night. The plants were watered as necessary and fertilized biweekly with Peters™ 20-20-20 General Purpose soluble fertilizer.

The CO_2-O_3 treatments were: 1) 400 ppm CO_2 and charcoal-filtered (CF) air, 2) 800 ppm CO_2 and CF air, 3) 400 ppm CO_2 and elevated O_3, and 4) 800 ppm CO_2 and elevated O_3. Ozone in the CF air was less than 0.05 ppm, while elevated O_3 was 0.15 ppm. The CO_2 exposures were continuous, whereas, the O_3 exposures were 6 hrs/day five days a week. CO_2 exposure began when the plants were placed in the chambers, and O_3 exposures started four days later. Using eight chambers, each treatment was replicated twice.

Measurements of net photosynthetic rate and stomatal conductance on initial (primary) leaves and leaves at nodes three or four were taken 28, 54, and 83 days after the seedlings were placed in the chambers. These measurements were taken at the CO_2 level to which plants were exposed during treatment. Net photosynthetic rates and stomatal conductances of leaves at nodes three and four could not be measured following 28 days of exposure because of insufficient leaf size. Data were analyzed using a one-way analysis of variance (ANOVA).

Carbon dioxide response curves were obtained by measuring net photosynthesis at different concentrations of CO_2 ranging from 50 to 700 ppm. Readings were taken after 35, 50, and 77 days. A linear regression model was calculated for each treatment data set. The slopes of the lines were compared with an analysis of covariance.

Seedlings were harvested after 85 days of treatment, and stem and leaf tissues were separated and dried at 70° C. Dry weights were determined and compared using an ANOVA.

RESULTS AND DISCUSSION

Significant differences in the net photosynthetic rate among the four treatments were found on each of the three measurement dates (Table 1). Without exception, net photosynthesis increased with an increase in the CO_2 concentrations or remained the same. The significant increases were observed on the initial leaves in both CF air and in elevated ozone treatments after 28 days of treatment,

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TABLE 1

Photosynthetic rates and stomatal conductance of initial leaves and leaves at nodes 3 or 4 after different exposure periods.

Days of Exposure	Treatment		Initial Leaves		Leaves of nodes 3 or 4	
	CO_2†	O_3†	Photosynthesis*	Conductance**	Photosynthesis	Conductance
28	400	CF ††	3.61b***	0.14a	—	—
	800	CF	5.46a	0.09b	—	—
	400	0.15	3.73b	0.13a	—	—
	800	0.15	6.32a	0.12ab	—	—
54	400	CF	1.10c	0.04bc	3.14ab	0.12a
	800	CF	3.80a	0.06ab	4.58a	0.09ab
	400	0.15	1.91bc	0.08a	2.57b	0.12a
	800	0.15	2.42b	0.02c	3.28ab	0.06b
83	400	CF	1.01ab	0.06a	2.96a	0.14a
	800	CF	1.87ab	0.04a	2.84a	0.08b
	400	0.15	0.74b	0.06a	1.67a	0.09ab
	800	0.15	2.35a	0.05a	3.85a	0.08b

†ppm
††CF = charcoal-filtered air (O_3 <0.05 ppm)
*umol CO_2 m^{-2} sec^{-1}
**mol m^{-2} sec^{-1}
***Values in the same column followed by same letter are not significantly different ($P = 0.05$).

in CF air after 54 days of treatment, and in elevated O_3 after 83 days of treatment. Net photosynthesis of the leaves at nodes 3 or 4 also tended to increase with increased CO_2 concentrations, but none of the trends was significant.

The increase in photosynthesis with increasing CO_2 has been observed by numerous authors (Dahlman et al. 1985, Allen 1990, Kramer 1981, Krupa and Kickert 1989, Norby and O'Neill 1989) on both agricultural and forest species. The increase results from the increased availability of CO_2 in the intercellular spaces of the leaves, but the mechanism of stimulation is unclear (Allen 1990). One hypothesis suggests that the impact of CO_2 on RUBP carboxylase activity causes the increase (Kramer and Sionit 1987). At ambient CO_2 levels there are excessive amounts of RUBP carboxylase in the leaves. As the partial pressure of CO_2 increases, more CO_2 is available for carboxylation. This is especially significant with regard to its influence on the photosynthetic rate since RUBP carboxylase has a relatively low affinity for CO_2 under ambient conditions.

The net photosynthetic response is also directly impacted by the stomatal response to elevated levels of CO_2. As observed in the present study, stomatal conductance is not always affected by elevated CO_2, but when significant differences were found, stomatal conductance was always reduced (Table 1) (Pearcy and Bjorkman 1983). The decrease in stomatal conductance reduced the rate of water vapor exchange, but probably not CO_2 exchange, between the intercellular spaces of the leaf and the atmosphere. Thus, the effect of stomatal conductance on CO_2 exchange may have been offset by the higher CO_2 partial pressure. Stomatal conductance correlated poorly with changes in net photosynthesis in this study since twice during the experiment photosynthesis increased with increasing levels of CO_2 while stomatal conductance decreased. This observation might be expected in view of the fact that stomata control transpiration in a linear fashion while affecting CO_2 exchange in a non-linear manner.

The objective of measuring photosynthesis repeatedly on the initial leaves and periodically on newly formed leaves at node 3 or 4 was to compare the effect of leaf aging in elevated CO_2 and O_3 with photosynthesis of newly formed leaves in the same atmosphere. The initial leaves continued to respond significantly to increased CO_2 with aging while the newly formed leaves did not.

Based on reports in the literature it was anticipated that the net photosynthetic rate of the initial leaves and the leaves at nodes 3 or 4 would be stimulated by elevated CO_2 (Allen 1990, Kramer and Sionit 1987) and as the experiment continued, that the net photosynthetic rate of the initial leaves in elevated CO_2 would fall. Such a reduction may be the result of feedback inhibition caused by reduced sink demand.

The lack of stimulation of net photosynthesis for the leaves at node 3 or 4 with elevated CO_2 after 54 and 83 days may have been in response to some acclimation process in the seedling. If the sink demands of the seedlings were being exceeded, the newer leaves may have had less RUBP carboxylase and, therefore, a lower photosynthetic capacity. Ozone had only a limited effect on net photosynthesis and stomatal conductance (Table 1). After 54 days of exposure, both parameters decreased with an increase in O_3 in elevated CO_2. This trend was also observed in ambient CO_2 with the leaves of nodes 3 and 4 after both 54 and 83 days. In CF air, elevated CO_2 may

have reduced or eliminated the impact of O_3 by stimulating net photosynthesis, thus providing enough photosynthate to: 1) repair any damage that ozone caused to the tissue, or 2) overcome the O_3 effect by reducing stomatal aperture, thus reducing the amount of O_3 entering the leaves.

Carbon dioxide did not have a significant effect on the shape of the CO_2 response curve throughout the experiment (Table 2). Ozone had a significant influence after 50 days of treatment in an atmosphere of 400 ppm CO_2.

The slope of the CO_2 response curve is a measure of the carboxylation efficiency and can be used to infer the relative activity of RUBP carboxylase (Sasek and Richardson 1989). Therefore, there was no difference in the RUBP carboxylase activity between the seedlings grown in 400 or 800 ppm CO_2.

TABLE 2

Slopes of regression base for CO_2 response curves.

Days of Exposure	Treatment CO_2*	Treatment O_3*	Slope
35	400	CF**	0.006a***
	800	CF	0.005a
50	400	CF	0.006a
	800	CF	0.007ab
	400	0.15	0.010b
	800	0.15	0.008ab
77	400	CF	0.010a
	800	CF	0.008a
	400	0.15	0.010a
	800	0.15	0.008a

*ppm
**CF = charcoal-filtered air (O_3 <0.05 ppm)
***Values followed by the same letter are not significantly different (P = 0.05)

Biomass increased with an increase in the CO_2 level in the atmosphere (Table 3). Leaf weight, stem weight, and root weight all increased except for root biomass in CF air. These results were similar to those reported by O'Neill et al. (1987). Ozone did not have a significant impact on any of the biomass variables; however, with elevated O_3 treatment, biomass increased with increasing CO_2.

The increase in growth was directly correlated with increased photosynthesis. The faster rate of net photosythesis would provide the larger amount of photosynthate needed for increased growth. However, a change in the net photosynthetic rate is not always correlated with a change in biomass or growth because net photosynthesis is an instantaneous measurement in time while biomass integrates the response of net photosynthesis and other physiological functions over a period of time.

TABLE 3

Dry weight of Acer saccharum *(gm) tissue.*

Treatment CO_2*	Treatment O_3*	Leaf	Stem	Root	Total
400	CF**	0.821bc***	0.285b	0.918ab	2.023b
800	CF	0.999ab	0.357ab	0.810b	2.165b
400	0.15	0.639c	0.233b	0.599b	1.471b
800	0.15	1.241a	0.418a	1.32a	2.987a

*ppm
**CF = charcoal-filtered air (O_3 <0.05 ppm)
***Values followed by the same letter are not significantly different (P = 0.05)

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